Growth Capacity in Response to Auxin of Coleoptile Segments of Normal and Dwarf Rice Strains

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Abstract. Auxin-induced growth, epidermal cell length, cellular osmotic potential, and cell wall composition of coleoptile segments excised from one normal and two dwarf rice strains were studied 2, 3, 4, and 5 days after soaking. The auxin-induced growth was higher at the early stages of coleoptile growth and decreased with age, being always higher in normal than in the two dwarf strains. A good correlation between auxin-induced growth and auxin-induced decrease in the minimum stress-relaxation time has been found, suggesting that the different growth capacity in response to auxin among the three different strains is due to differences in the structure of their cell walls. In fact, cell wall analysis revealed that (1) the relative α-cellulose content of the cell walls was higher in the two dwarf strains than in the normal one, and (2) the auxin-induced decrease in noncellulosic glucose was high, compared with dwarf strains, in the normal strain, which showed the higher auxin-induced growth, showing a highly significant correlation between the decrease in noncellulosic glucose and the growth in response to auxin. Thus, the different growth between normal and dwarf strains might be attributed to their different capacity to degrade β-glucan of their cell walls.

Most physiological studies on dwarfism have focused on gibberellin deficiencies (Murakami 1968, Suge and Murakami 1968, Harada and Vergara 1972, Singh et al. 1981, Singh and Singh 1982). However, gibberellins do not overcome the stunted growth of dwarf wheat coleoptiles (Ricard and Nitsch 1958)

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and dwarf barley coleoptiles (Suge 1972, Kuraishi 1974). Furthermore, the growth of etiolated intact plants (Lockhart 1958, Lockhart and Gottschall 1959, Wakhloo 1975) or internode sections (Jones 1983) is insensitive to exogenously applied gibberellins. These results suggest that gibberellin deficiencies may not be the only cause of dwarfism in coleoptiles grown in the dark. Studies with different strains of barley coleoptiles grown in the dark have shown that dwarfism is due to the endogenous auxin content (Inouhe et al. 1982) in the early growth stage and that the chemical structure of the cell wall (Sakurai et al. 1983) and its mechanical properties (Sakurai et al. 1984) could be the controlling factor at further stages of coleoptile development.

Since our previous studies on rice coleoptile growth (cv. Nihonbare) showed that coleoptile growth could be controlled by two main factors—cellular osmotic potential and cell wall composition (Zarra and Masuda 1971a,b)—we have analyzed these factors during coleoptile development using three rice strains: one normal (cv. Nihonbare) and two dwarfs (cv. Tan-ginbozu and Waito C.).

Materials and Methods

Seeds of three rice strains (*Oryza sativa* L.), one normal (Nihonbare) and two dwarf strains (Tan-ginbozu and Waito C), were soaked in water for 2 days, germinated, and grown on moistened cotton at 25°C in the dark, as described in a previous paper (Zarra and Masuda 1971a).

Four-millimeter subapical coleoptile segments were excised at different coleoptile ages and starved in 10 mM potassium citrate phosphate buffer solution (pH 6.5) for 3 h at 25°C in the dark, changing the buffer solution every hour. The epidermal cell length of the starved segments was measured to the nearest 2.5 μm with a microscope equipped with an ocular micrometer.

To determine the osmotic potential of the coleoptile cells, 20 starved coleoptile segments were centrifuged at 1,000g for 5 min to remove intercellular solution, then wrapped in aluminum foil and frozen with liquid nitrogen. After 18 h they were thawed at room temperature, and the cell sap was obtained by centrifugation at 1,000g, for 10 min, as developed by Terry and Bonner (1980) and modified by Zhao et al. (1983). The osmotic potential of cell sap was determined with a Vapor Pressure Osmometer (Wescor model 5100 C) and calculated according the formula $\pi = -RCT$, where π is osmotic potential, R is gas constant, T is absolute temperature and C is concentration.

The starved segments were transferred to a small Petri dish containing 4 ml of buffer solution with or without 5×10^{-5} M IAA and incubated for 5 h at 25°C in the dark. The final segment length was measured to the nearest 0.15 mm with a binocular microscope equipped with an ocular micrometer.

After completion of the growth measurements, the coleoptile segments were immediately fixed in boiling methanol. The rehydrated segments were treated with 200 ppm pronase (Kaken Kagaku Co.) for 18 h at 37°C. After pronase treatment, the mechanical properties of the coleoptile cell wall were measured by an Instron technique as described in Zarra and Masuda (1979a), and the minimum stress-relaxation time (T_0) was calculated as described by Yamamoto et al. (1974).

Cell walls of coleoptile segments whose mechanical properties had been determined were prepared, and the amounts of neutral noncellulosic sugars and α-cellulose were determined as described previously (Zarra and Masuda 1979a).

Results

Coleoptile Growth

Figure 1 shows the coleoptile growth curves of the three different rice strains. The maximum coleoptile length was achieved by the normal strain (cv. Nihonbare), 24.2 ± 0.6 mm at the fifth day after soaking, whereas values of 18.8 ± 0.5 mm and 17.0 ± 0.6 mm were obtained for Tan-ginbozu and Waito C, respectively. A fast growth phase was observed after the second day for the three strains, the growth rate being larger for the Nihonbare strain. While the Nihonbare coleoptile grows at a constant rate until the fifth day, growth rate for both Tan-ginbozu and Waito C decreased, resulting the difference in the final coleoptile length between normal and dwarf strains.

The cell number in a longitudinal row along the coleoptile at day 5, estimated by dividing the coleoptile length by the cell length, was 139 ± 18, 148 ± 16, and 107 ± 15 for Nihonbare, Tan-ginbozu, and Waito C, respectively. Thus, the difference in the coleoptile length among the three strains cannot be simply explained in terms of cell number.

Growth in Response to Auxin

Auxin-induced growth (Fig. 2) was maximum at day 2, when the epidermal cell length was shorter. Auxin response decreased with the age, whereas the epidermal cell length increased. When the growth capacity in response to auxin in the same age for the three strains was compared, it was always higher for the normal strain (cv. Nihonbare) than for the two dwarf strains (cv. Tanginbozu and Waito C). The supplementary addition of 10^{-4} M gibberellic acid (data not shown) to the incubation medium did not change the auxin-induced growth of coleoptile segments from any strain. Thus, the lower response to auxin of the dwarf coleoptile segments was apparently not due to a low level in endogenous gibberellins.

Osmotic Potential

Since the osmotic potential has been considered to be the driving force of plant cell elongation (Cleland 1971, Masuda 1978), we also studied the osmotic potential of the coleoptile cells in the three different strains (Table 1). The osmotic potential reached the maximum value in the three strains at day 3 after soaking, when the cell division in the coleoptile was completed (Wada 1961). Afterward, the osmotic potential slightly decreased until day 5. Although there were significant differences among the three strains, they were not correlated with the differences in the growth response to auxin.
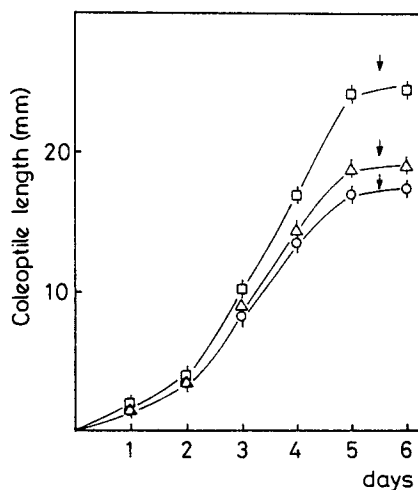
Fig. 1. Growth curve of rice coleoptiles from three different strains. □, Nihonbare; △, Tan-ginbozu; ○, Waito C. Every day, 1–6 days after soaking, the coleoptile length was measured. Arrows indicate the times when the primary leaves emerged from the coleoptile. Mean value of 20 coleoptiles with standard errors (vertical lines).
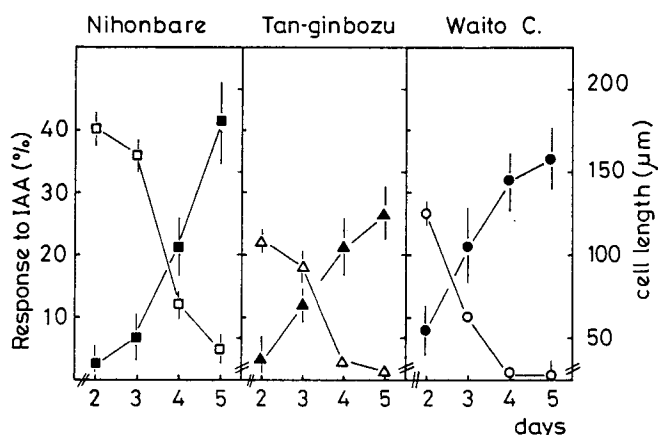

Fig. 2. Growth response to IAA and initial epidermal cell length of coleoptile segments at days 2, 3, 4, and 5 from the three rice strains. Segments were starved for 3 h in a buffer solution and then incubated for 5 h in a medium containing or not 5×10^{-5} M IAA. Growth was expressed as $(L_{IAA} - L_C)/(L_0) \times 100$, L_{IAA} and L_C being final length after incubation with or without IAA, respectively; and L_0, length after starvation. □ ■, Nihonbare; △ ▲, Tan-ginbozu; ○ ●, Waito C. Open symbols, growth response; solid symbols, epidermal cell length. Mean of 20 segments and 100 cells with standard errors (vertical lines).

Mechanical Properties of the Cell Wall

The mechanical properties of the cell wall of the coleoptile segments excised at different ages in tall and dwarf rice strains were measured in order to examine the effect of auxin on cell wall loosening (Table 2). Indole-3-acetic acid (IAA) significantly decreased the T_0 value of coleoptile segments from 2- and 3-day seedlings of the three different rice strains, which showed auxin-induced growth. However, auxin did not induce changes in the T_0 value of older co-

Table 1. Cellular osmotic potential of starved coleoptile segments excised at days 2, 3, 4, and 5 of the three rice strains

Age (days)	Osmotic potential, -atm.		
	Nihonbare	Tan-ginbozu	Waito C
2	6.3 ± 0.2	6.1 ± 0.1	6.4 ± 0.1
3	7.0 ± 0.1	7.0 ± 0.1	6.7 ± 0.1
4	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1
5	6.3 ± 0.1	6.2 ± 0.1	6.5 ± 0.1

Mean value and standard errors of five samples.

Table 2. Effect of IAA on minimum stress-relaxation time (T_0) of coleoptile segments from three rice strains excised at days 2, 3, 4, and 5 after soaking

Age (days)	T_0 (msec)			
	2	3	4	5
Nihonbare				
Initial	24.2 ± 1.3	26.5 ± 2.9	34.3 ± 2.1	35.2 ± 1.9
+ IAA	20.5 ± 1.2	21.2 ± 1.8	32.1 ± 1.9	37.8 ± 1.6
− IAA	23.2 ± 1.2	33.4 ± 2.7	35.3 ± 2.3	38.4 ± 4.3
Tan-ginbozu				
Initial	26.4 ± 1.5	27.8 ± 1.8	35.3 ± 1.9	35.2 ± 2.0
+ IAA	23.7 ± 1.7	24.1 ± 1.6	34.9 ± 2.6	34.3 ± 2.1
− IAA	27.2 ± 1.2	28.4 ± 1.4	36.3 ± 2.2	35.3 ± 2.1
Waito C				
Initial	25.9 ± 1.6	28.9 ± 1.9	34.2 ± 1.9	34.9 ± 2.4
+ IAA	22.8 ± 1.2	26.5 ± 1.5	34.3 ± 2.0	33.8 ± 2.1
− IAA	25.2 ± 1.1	28.4 ± 1.7	35.7 ± 1.7	35.6 ± 2.4

Mean values with standard errors of twenty samples are given. See legend to Fig. 2.

leoptiles, which did not respond to auxin. These results confirm a good correlation between auxin-induced growth and auxin-induced decrease in the T_0 value, being in agreement with data obtained for other graminaceous coleoptiles (Sakurai and Masuda 1977, Zarra and Masuda 1979b).

Cell Wall Composition

Holocellulose constituted more than 80% of the cell wall dry weight and showed no significant changes during coleoptile growth in the three rice strains. However, the amount of α-cellulose showed significant differences between normal and dwarf strains (Fig. 3), the α-cellulose increase with the coleoptile age being more pronounced in the two dwarf strains. The α-cellulose content of Nihonbare cell wall was always lower than the amount of neutral noncellulosic sugars even at days 4 and 5, when coleoptile segments did not grow in response to auxin. However, the α-cellulose amount of Tan-ginbozu and Waito C strains, which at day 2 was lower than the amount of neutral noncellulosic
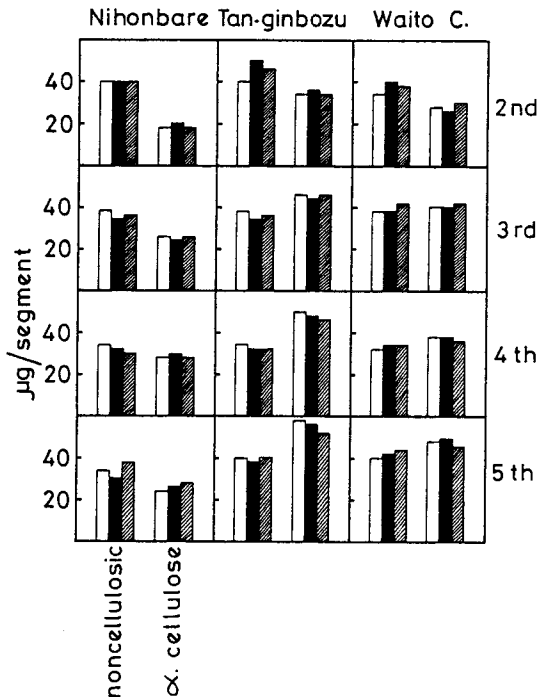
Fig. 3. Auxin-induced changes in cell wall components of rice coleoptile segments at different ages, incubated as in Fig. 2. Open bars, starved segments; solid bars, IAA-treated segments; hatched bars, control segments.

sugars, became higher after day 3, when the coleoptile segments of that strains did not respond to auxin. Thus, it is clear that the coleoptile segments of the dwarf strains that showed a lower response to auxin have a higher relative amount of α-cellulose.

Figure 4 shows the neutral noncellulosic sugar composition of the coleoptile segments and their changes during auxin-induced growth. The main sugars were arabinose, xylose, galactose, and glucose, as has been found in coleoptiles of other monocots (Sakurai and Masuda 1977, Sakurai et al. 1983). Trace amounts of mannose, rhamnose, and fucose were also found. The more conspicuous change in neutral noncellulosic sugar composition was in the amount of noncellulosic glucose, which decreases with the coleoptile age and also during the auxin-induced growth. The auxin-induced decrease in noncellulosic glucose was large in segments from 2- and 3-day-old coleoptiles when the growth in response to auxin was also large. The auxin-induced glucose decrease was less conspicuous in dwarf coleoptile segments, which elongate less in response to added auxin. It also appears that the increase in the amount of xylose with age was larger in the dwarf coleoptiles than in the normal coleoptiles.

That there is a continuous decrease in the amount of noncellulosic glucose of the cell wall in normal and dwarf strains of rice coleoptiles during intact growth and also during auxin-induced growth agrees with the hypothesis postulated by Nevins (1975) and Sakurai and Masuda (1977, 1978) that cell wall loosening in monocot coleoptiles is achieved through a mechanism involving a partial degradation of a cell wall β-glucan.
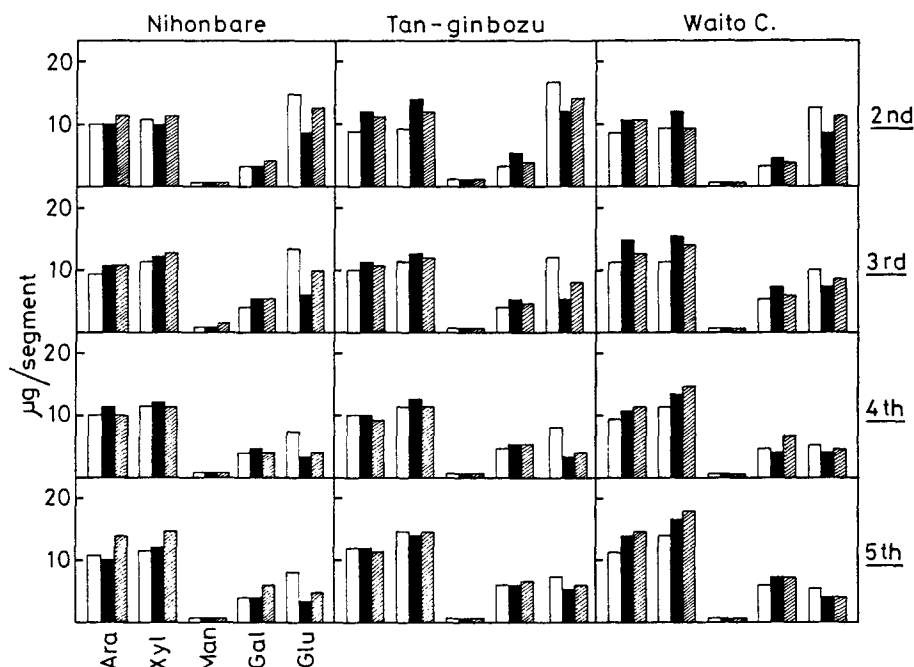
Fig. 4. Auxin-induced changes in sugar composition of noncellulosic neutral sugars of cell wall of rice coleoptile segments at different ages. Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose. See the legend to Fig. 3.

Discussion

As shown in Figs. 1 and 2, the final coleoptile length and the auxin-induced growth of coleoptile segments were lower in dwarf (cv. Tan-ginbozu and Waito C) than in normal strain (cv. Nihonbare) of etiolated rice seedlings. That the lower response to auxin of dwarf coleoptile segments was not overcome by simultaneous addition of auxin and gibberellic acid suggests that phytohormone deficiency is not a main factor causing dwarfism in rice seedlings but that some other unknown factors are involved.

The auxin-induced growth of the coleoptile segments in the three strains was strongly correlated with their epidermal cell length (Fig. 5). The correlation coefficient is -0.87, being significant at the 99% level. It thus seems clear that the lower response to auxin of dwarf strains might be caused by the different extension capacity of their cells.

The osmotic potential and the cell wall extension capacity are the main factors controlling the cell elongation (Cleland 1971, Masuda 1978). The differences among the osmotic potential of the cells in the three strains cannot explain their different responses to auxin (Table 1). However, the auxin-induced changes in the cell wall mechanical properties showed a good correlation with the auxin-induced growth.

Since a decrease in the minimum stress-relaxation time has been reported to represent the degree of cell wall loosening (Masuda 1978), these results
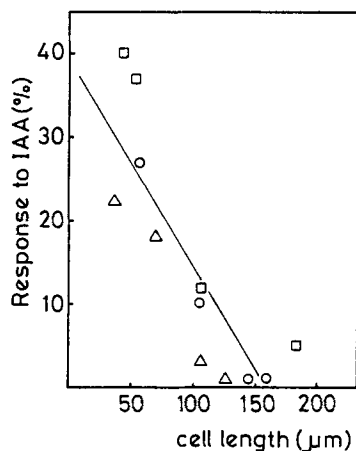
Fig. 5. Correlation between growth in response to auxin and epidermal cell length of starved coleoptile segments. See legend to Fig. 2.
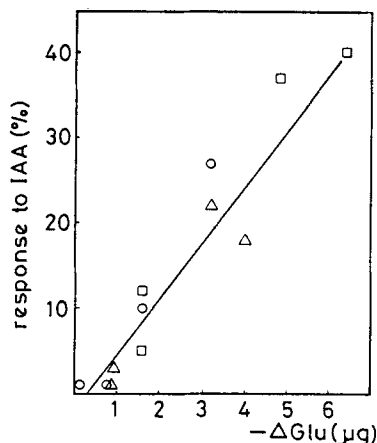

Fig. 6. Correlation between growth in response to auxin and auxin-induced decrease in noncellulosic glucose. See legend to Fig. 2.

suggest that the growth differences between the normal and dwarf strains could be explained in terms of their cell wall composition. The higher amount of α-cellulose, a stiffening component of the cell wall (Masuda et al. 1974) in the dwarf coleoptile segments, is in agreement with the lower effect of auxin on both growth and mechanical properties.

A highly significant correlation (at a 99.5% level) between auxin-induced decrease in noncellulosic glucose and auxin-induced growth has been found (Fig. 6), indicating that the growth response to auxin of the three strains at their different growth stages is due to a different capacity to degrade a non-cellulosic, glucose-rich polysaccharide.

Main hemicellulosic components of rice seedlings have been reported to be β-1,3,β-1,4-glucan, arabinoxylan, and xyloglucan (Kato et al. 1982). In addition, there is evidence that exogenously applied IAA to excised rice coleoptile segments induced growth and caused a decrease in β-glucan (Zarra and Masuda 1979b), as auxin does in oat (Sakurai and Masuda 1977) and barley (Sakurai

and Masuda 1978) coleoptile segments. Thus, the lower growth response to auxin of dwarf coleoptile segments seems to be due to a lower capacity of their cells to degrade the β-glucan of their cell walls. Since the auxin-induced growth is only correlated with auxin-decrease in noncellulosic glucose and it is not with their initial level in the cell wall, the difference between normal and dwarf strains might, at least partly, be attributed to their different enzymatic systems or activities of β-glucanase, as it has been postulated by Sakurai and Kuraishi (1984) for dwarf barley coleoptiles.

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